L3: Entry 13 of 17 File: USPT Jul 14, 1998

DOCUMENT-IDENTIFIER: US 5780289 A
TITLE: Coccidiosis poultry vaccine DNA encoding an elmeria 20K antigen

Abstract Paragraph Left (1):
The invention is concerned with novel Eimeria proteins with immunogenic properties as well as with DNA sequences encoding these proteins. These proteins can be administered to chickens thereby protecting the chickens against coccidiosis. In addition the DNA encoding these proteins can be used for the preparation of a vector vaccine against coccidiosis.

Parent Case Paragraph Right (2):
The present invention is concerned with a protein having one or more immunogenic determinants of an Eimeria antigen, a nucleic acid sequence encoding this protein, a recombinant vector molecule or recombinant vector virus comprising such a nucleic acid sequence, a host cell transformed with such a recombinant vector molecule or infected with the recombinant vector virus, antibodies immuno-reactive with said protein, as well as a vaccine for the protection of avians against coccidiosis.

Brief Summary Paragraph Right (1):
Coccidiosis is a disease which is caused by intracellular parasites, protozoa, of the subphylum Apicomplexa and the genus Eimeria. These parasites multiply in cells which form part of the gastro-intestinal tract and digestive organs.

Brief Summary Paragraph Right (3):
The pathogens of coccidiosis in chickens can be subdivided into nine different species, i.e. Eimeria acervulina, E. maxima, E. tenella, E. necatrix, E. brunetti, E. mitis, E. praecox, E. mivati and E. hagani. However, some people doubt the existence of the last two species. All of these species have only the chicken as host and display a high degree of tissue specificity. The life cycles of the said species are, however, similar.

Brief Summary Paragraph Right (5):
During the life cycle, the Eimeria parasites pass through a number of stages. The infectious stage (the sporulating oocyst) is taken in orally and passes into the stomach of the chicken, where the wall of the cyst bursts open as a result of the grinding action. The four sporocysts, which this oocyst contains, are released and pass into the duodenum, where they are exposed to bile and digestive enzymes. As a result, an opening is made in the sporocyst wall and the sporozoites present in the sporocyst are released. These sporozoites are mobile and search for suitable host cells, epithelium cells, in order to penetrate and to reproduce. Depending on the species, this first reproduction phase lasts 20 to 48 hours and several tens to hundreds of merozoites are formed, which each again penetrate a new host cell and reproduce. After two to sometimes five of these asexual reproduction cycles, depending on the species the intracellular merozoites grow into sexual forms, the male and female gametocytes. After fertilization of the female by a male gamete, a zygote is formed which creates a cyst wall about itself. This oocyst leaves the host cell and is driven out with the faeces. If the temperature and humidity outside the chicken are relatively high and, at the same time, there is sufficient oxygen in the air, the oocyst can sporulate to the infectious stage.

Brief Summary Paragraph Right (9):
Immunological prophylaxis would, therefore, constitute a much better combatting

method. It is known that chickens which have lived through a sufficiently high infection are able to resist a subsequent contact with the same type of Eimeria. Resistance towards Eimeria can also be induced by infecting the birds several times with low doses of oocysts or with oocysts of weakened (non-pathogenic) strains. However, controlled administration to, specifically, large numbers of broiler chickens is a virtually insurmountable problem in this case.

Brief Summary Paragraph Right (10):
According to the present invention purified proteins having one or more immunogenic determinants of an Eimeria antigen, essentially free from the whole parasite or other protein with which they are ordinarily associated are provided which can be used for the preparation of a vaccine for the immunization of avians, in particular poultry against coccidiosis.

Drawing Description Paragraph Right (1):
FIG. 1A & B is a panel of different Eimeria species and stages reacting with monoclonal antibodies E.ACER 11A-2A (Panel A) and E.ACER 12B-2B (Panel B).

Drawing Description Paragraph Right (2):
FIG. 2A & B is a panel of different Eimeria species and stages reacting with monoclonal antibodies E.ACER 10C-2A (Panel A) and E.ACER 10E-2 (Panel B).

Detailed Description Paragraph Right (3):
The term "protein having one or more immunogenic determinants of an Eimeria antigen" refers to a protein having one or more epitopes capable of eliciting an immune response against Eimeria parasites in host animals.

Detailed Description Paragraph Right (5):
In particular, the invention provides proteins having one or more immunogenic determinants of an Eimeria antigen wherein the Eimeria antigen has a molecular weight in SDS-PAGE of about 200, 100, 50 or 20 kD and the Eimeria antigen specifically binds with monoclonal antibody E.ACER 11A-2A or E.ACER 12B-2B, E.ACER 5F-2, E.ACER 10C-2A or E.ACER 10E-2, respectively. Samples of the hybridoma cell lines producing these monoclonal antibodies were deposited with the European Collection of Animal Cell Cultures (ECACC) at Porton Down, UK, under the accession No. 91061223 (E.ACER 12B-2B), 91061222 (E.ACER 11A-2A), 91061219 (E.ACER 5F-2), 91061220 (E.ACER 10C-2A) and 91061221 (E.ACER 10E-2).

Detailed Description Paragraph Right (6):
The Eimeria antigens disclosed above can be characterized by their isolation procedure, i.e. the antigens are obtainable by:

Detailed Description Paragraph Right (7):
Preferred proteins according to the invention comprise one or more immunogenic determinants of the Eimeria acervulina antigens Eam200, Eam100 or Eas100, Eam45 or Eam20 (Example 2).

Detailed Description Paragraph Right (8):
Eam200 is an Eimeria protein of about 200 kD purified from Eimeria acervulina merozoites and is immuno-reactive with monoclonal antibody (Mab) E.ACER 11A-2A.

Detailed Description Paragraph Right (9):
Eas100 is an Eimeria protein of about 100 kD purified from Eimeria acervulina sporozoites and is immuno-reactive with Mab E.ACER 5F-2, Eam100 is the merozoite equivalent.

Detailed Description Paragraph Right (10):
Eam45 is an Eimeria protein of about 50 kD purified from Eimeria acervulina merozoites and is immuno-reactive with Mab E.ACER 10C-2A.

Detailed Description Paragraph Right (11):
Eam20 is an Eimeria protein of about 20 kD purified from Eimeria acervulina merozoites and is immuno-reactive with Mab E.ACER 10E-2.

Detailed Description Paragraph Right (19):

More particularly, this invention provides examples of proteins having one or more immunogenic determinants of the purified Eimeria antigens identified above. These examples are proteins comprising the amino acid sequence shown in SEQ ID NO.: 2, 6, 8 or 10 and its functional variants.

Detailed Description Paragraph Right (20):
In addition, the present invention provides an Eimeria protein having the amino acid sequence shown in SEQ ID NO. 4 and its functional variant. This protein was identified by screening an Eimeria merozoite cDNA library with anti-Eam45 serum. This serum demonstrated a positive reaction with an about 100 kD protein (in addition to a positive reaction with the about 50 kD protein) when probing this serum back on a merozoite blot (FIG. 9).

Detailed Description Paragraph Right (21):
The functional variants of the proteins specifically disclosed herein are proteins derived from the above-noted amino acid sequences, for example by deletions, insertions and/or substitutions of one or more amino acids, but retain one or more immunogenic determinants of the Eimeria antigens, i.e. said variants have one or more epitopes capable of eliciting an immune response in a host animal.

Detailed Description Paragraph Right (22):
It will be understood that for the particular proteins embraced herein, natural variations can exist between individual Eimeria parasites or strains. These variations may be demonstrated by (an) amino acid difference(s) in the overall sequence or by deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said sequence. Amino acid substitutions from which can be expected that they do not essentially alter biological and immunological activities, have been described. Amino acid replacements between related amino acids or replacements which have occurred frequently in evolution are, inter alia Ser/Ala, Ser/Gly, Asp/Gly, Asp/Asn, Ile/Val (see Dayhof, M. D., Atlas of protein sequence and structure, Nat. Biomed. Res. Found., Washington D.C., 1978, vol. 5, suppl. 3). Based on this information Lipman and Pearson developed a method for rapid and sensitive protein comparison (Science 227, 1435-1441, 1985) and determining the functional similarity between homologous proteins.

Detailed Description Paragraph Right (24):
The term "fragment" as used herein means a DNA or amino acid sequence comprising a subsequence of the nucleic acid sequence or protein of the invention. Said fragment is or encodes a polypeptide having one or more immunogenic determinants of an Eimeria antigen. Methods for determining usable immunogenic polypeptide fragments are outlined below. Fragments can inter alia be produced by enzymatic cleavage of precursor molecules, using restriction endonucleases for the DNA and proteases for the polypeptides. Other methods include chemical synthesis of the fragments or the expression of polypeptide fragments by DNA fragments.

Detailed Description Paragraph Right (28):
The invention further provides isolated and purified nucleic acid sequences encoding the above-noted proteins of Eimeria.

Detailed Description Paragraph Right (31):
The information provided in SEQ ID NO's: 1, 3, 5, 7 and 9 allows a person skilled in the art to isolate and identify the nucleic acid sequences encoding the various functional variant proteins mentioned above having corresponding immunological characteristics with the Eimeria proteins specifically disclosed herein. The generally applied Southern blotting technique or colony hybridization can be used for that purpose (Experiments in Molecular Biology, ed. R. J. Slater, Clifton, U.S.A., 1986; Singer-Sam, J. et al., Proc. Natl., Acad. Sci. 80, 802-806, 1983; Maniatis T. et al., Molecular Cloning, A laboratory Manual, second edition, Cold Spring Harbor Laboratory Press, USA, 1989). For example, a CDNA library derived from a specific Eimeria strain is transferred, or "blotted" onto a piece of nitrocellulose filter. It is now possible to identify specific Eimeria nucleic acid sequences on the filter by hybridization to a defined labeled DNA fragment or "probe", i.e. a (synthetic) poly- or oligonucleotide sequence derived from the nucleic acid sequence shown in SEQ ID NO's: 1, 3, 5, 7 and 9, which under specific conditions of salt concentration and temperature hybridizes to the homologous nucleic acid sequences present on the filter. After washing the filter, hybridized material may be detected by autoradiography. The corresponding DNA fragment

can now be eluted from the agarose gel and used to direct the synthesis of a functional variant of the polypeptide disclosed in SEQ ID NO's: 2, 4, 6, 8 or 10.

Detailed Description Paragraph Right (32):
Typically, a CDNA library from Eimeria can be constructed exactly according to the procedure described in Example 3. The inserts from clones pGEM4Z Eam200, pGEM4Z Eam45 M1(E), pGEM4Z Eam45 M3(E) pGEM4Z Eam20(E) or pGEM4Z Eam100E can be labeled with digoxigenin-dUTP by random priming, exactly following the protocol going with the "DNA labelling and detection kit, non-radioactive" from Boehringer, Mannheim (Cat. No. 1093657).

Detailed Description Paragraph Right (33):
Filters containing immobilized DNA from the Eimeria cDNA library described above can be prepared as described by Maniatis et al., supra and probed by the freshly denatured (10 min.,95.degree. C.), labeled Eimeria fragment for 16 hours at 42.degree. C. according to the manufacturer's instructions. Filters are then washed as follows: twice for fifteen minutes with 2.times.SSC, 0.1% (w/v) SDS (1.times.SSC is 0.015 mol/l sodium citrate pH 7.0 plus 0.15 mol/l NaCl) at room temperature and twice for fifteen minutes with 1.times.SSC, 0.1% (w/v) SDS at 55.degree. C. For final identification filters are then washed twice with PBS-tween (7.65 g/l NaCl, 0.91 g/l Na.sub.2 HPO.sub.4.2H.sub.2 O, 0.21 g/l KH.sub.2 PO.sub.4, 0.05% (v/v) Tween 80, pH 7.3) for 15 minutes at room temperature. The filters were then reacted with a 1:5000 dilution in PBS-tween of polyclonal sheep anti-digoxigenin Fab-fragments, conjugated to alkaline phosphatase, for thirty minutes at room temperature. After washing the filters for four times fifteen minutes with PBS-tween at room temperature and once for fifteen minutes with 0.01M Tris-HCl pH 8.0, 0.15M NaCl, binding of the alkaline phosphatase to the filters was detected upon incubation with a solution of 0.33 g/l Nitroblue tetrazolium and 0.17 g/l 5-bromo-4-chloro-3-indolyl-phosphate in 0.1M Tris-HCl pH 9.6, 0.1M NaCl, 0.01M MgCl.sub.2. The DNA that reacts with the probe can be used to express the encoding polypeptide as outlined below.

Detailed Description Paragraph Right (34):
Thereafter, the polypeptide can be assayed for the presence of one or more immunogenic determinants of an Eimeria antigen protein according to one of the following methods.

Detailed Description Paragraph Right (36):
Another possibility to achieve this is the antibody select technique binding antibodies directly to a filter containing a monoculture of recombinant phages in E. coli expressing the Eimeria DNA insert. By eluting these bound antibodies using the procedure of osaki et al (J. Immunological Methods 89, 213-219, 1986) and allowing them to bind again to Western blots of Eimeria antigens the connection is a fact. The latter procedure was followed for the Eas100 and the Eam45 clones (Example 3, FIGS. 8 and 9).

Detailed Description Paragraph Right (38):
Therefore, a nucleic acid sequence encoding a functional variant of the proteins disclosed herein encodes a polypeptide comprising one or more immunogenic determinants of an Eimeria antigen and hybridizes to the DNA sequence shown in SEQ ID NO's: 1, 3, 5, 7 or 9.

Detailed Description Paragraph Right (39):
In another way Eimeria cDNA may be cloned into a .lambda.gt11 phage as described by Huynh et al. (In: D. Glover (ed.), DNA Cloning: A Practical Approach, IRL Press Oxford, 49-78, 1985) and expressed into a bacterial host. Recombinant phages can then be screened with polyclonal serum raised against the purified Eimeria proteins described above or in SEQ ID NO's: 2, 4, 6, 8 or 10 determining the presence of corresponding immunological regions of the variant polypeptide. The production of the polyclonal serum to be used herein elicited against the Eimeria proteins is described below.

Detailed Description Paragraph Right (40):
More particularly, the present invention comprises nucleic acid sequences encoding a protein having one or more immunogenic determinants of an Eimeria antigen, wherein the nucleic acid sequences contain at least part of the DNA sequences shown in SEQ ID NO's: 1, 3, 5, 7 or 9, respectively.

Detailed Description Paragraph Right (41):
A nucleic acid sequence according to the invention may be isolated from a particular Eimeria strain and multiplied by recombinant DNA techniques including polymerase chain reaction (PCR) technology or may be chemically synthesized in vitro by techniques known in the art.

Detailed Description Paragraph Right (51):
For expression nucleic acid sequences of the present invention are introduced into an expression vector, i.e. said sequences are operably linked to expression control sequences. Such control sequences may comprise promoters, enhancers, operators, inducers, ribosome binding sites etc. Therefore, the present invention provides a recombinant vector molecule comprising a nucleic acid sequence encoding an Eimeria protein identified above operably linked to expression control sequences, capable of expressing the DNA sequences contained therein in (a) transformed host cell(s).

Detailed Description Paragraph Right (52):
It should, of course, be understood that the nucleotide sequences inserted at the selected site of the cloning vector may include nucleotides which are not part of the actual structural gene for the desired polypeptide or may include only a fragment of the complete structural gene for the desired protein as long as transformed host will produce a polypeptide having at least one or more immunogenic determinants of an Eimeria antigen.

Detailed Description Paragraph Right (53):
When the host cells are bacteria, illustrative useful expression control sequences include the Trp promoter and operator (Goeddel, et al., Nucl. Acids Res. 8, 4057, 1980); the lac promoter and operator (Chang, et al., Nature 275, 615, 1978); the outer membrane protein promoter (Nakamura, K. and Inouge, M., EMBO J. 1, 771-775, 1982); the bacteriophage .lambda.promoters and operators (Remaut, E. et al., Nucl. Acids Res. 11, 4677-4688, 1983); the .alpha.-amylase (B. subtilis) promoter and operator, termination sequence and other expression enhancement and control sequences compatible with the selected host cell. When the host cell is yeast, illustrative useful expression control sequences include, e.g., .alpha.-mating factor. For insect cells the polyhedrin or p10 promoters of baculoviruses can be used (Smith, G. E. et al., Mol. Cell. Biol. 3, 2156-65, 1983). When the host cell is of mammalian origin illustrative useful expression control sequences include, e.g., the SV-40 promoter (Berman, P. W. et al., Science 222, 524-527, 1983) or, e.g. the metallothionein promoter (Brinster, R. L., Nature 296, 39-42, 1982) or a heat shock promoter (Voellmy et al., Proc. Natl. Acad. Sci. USA 82, 4949-53, 1985). Alternatively, also expression control sequences present in Eimeria may be applied. For maximizing gene expression, see also Roberts and Lauer (Methods in Enzymology 68, 473, 1979).

Detailed Description Paragraph Right (54):
Therefore, the invention also comprises (a) host cell(s) transformed with a nucleic acid sequence or recombinant expression vector molecule described above, capable of producing the Eimeria protein by expression of the nucleic acid sequence.

Detailed Description Paragraph Right (55):
Immunization of avians against Eimeria infection can, for example be achieved by administering to the animals a protein according to the invention in an immunologically relevant context as a so-called subunit vaccine. The subunit vaccine according to the invention may comprise a protein in a pure form, optionally in the presence of a pharmaceutically acceptable carrier. The protein can optionally be covalently bonded to a non-related protein, which, for example can be of advantage in the purification of the fusion product. Examples are .beta.-galactosidase, protein A, prochymosine, blood clotting factor Xa, etc.

Detailed Description Paragraph Right (57):
Proteins to be used in such subunit vaccines can be prepared by methods known in the art, e.g. by isolating said polypeptides from Eimeria parasites, by recombinant DNA techniques or by chemical synthesis.

Detailed Description Paragraph Right (61):
Furthermore, the invention also comprises (a) host cell(s) or cell culture infected

with the recombinant vector virus, capable of producing the Eimeria protein by expression of the nucleic acid sequence.

Detailed Description Paragraph Right (68):
Host cells transformed with a recombinant vector molecule according to the invention can also be cultured under conditions which are favourable for the expression of a polypeptide coded by said nucleic acid sequence. Vaccines may be prepared using samples of the crude culture, host cell lysates or host cell extracts, although in another embodiment more purified polypeptides according to the invention are formed to a vaccine, depending on its intended use. In order to purify the polypeptides produced, host cells transformed with a recombinant vector according to the invention are cultured in an adequate volume and the polypeptides produced are isolated from such cells or from the medium if the protein is excreted. Polypeptides excreted into the medium can be isolated and purified by standard techniques, e.g. salt fractionation, centrifugation, ultrafiltration, chromatography, gel filtration or immuno affinity chromatography, whereas intra cellular polypeptides can be isolated by first collecting said cells, disrupting the cells, for example by sonication or by other mechanically disruptive means such as French press followed by separation of the polypeptides from the other intra cellular components and forming the polypeptides to a vaccine. Cell disruption could also be accomplished by chemical (e.g. EDTA or detergents such as Triton X114) or enzymatic means such as lysozyme digestion.

Detailed Description Paragraph Right (70):
The Eimeria proteins as characterized above can be used to produce antibodies, both polyclonal, monospecific and monoclonal. If polyclonal antibodies are desired, techniques for producing and processing polyclonal sera are known in the art (e.g. Mayer and Walter, eds, Immunochemical Methods in Cell and Molecular Biology, Academic Press, London, 1987). In short, a selected mammal, e.g. rabbit is given (multiple) injections with above-mentioned immunogens, about 20 .mu.g to about 80 .mu.g of protein per immunization. Immunizations are given with an acceptable adjuvant, generally equal volumes of immunogen and adjuvant. Acceptable adjuvants include Freund's complete, Freund's incomplete, alum-precipitate or water-in-oil emulsions, with Freund's complete adjuvant being preferred for the initial immunization. Freund's incomplete adjuvant is preferred for all booster immunizations. The initial immunization consists of the administration of about 1 ml of emulsion at multiple subcutaneous sites on the backs of the rabbits. Booster immunizations utilizing an equal volume of immunogen are given at about one month intervals and are continued until adequate levels of antibodies are present in an individual rabbits serum. Blood is collected and serum isolated by methods known in the art.

Detailed Description Paragraph Right (72):
Monoclonal antibody reactive against the Eimeria immunogens can be prepared by immunizing inbred mice, preferably Balb/c with the appropriate protein. The mice are immunized intraperitoneally with about 100 ng to about 10 .mu.g immunogen per 0.5 ml dose in an equal volume of an acceptable adjuvant. Such acceptable adjuvants include Freund's complete, Freund's incomplete, alum-precipitate and water-in-oil emulsions. The mice are given intravenous booster immunizations of an equal amount of the immunogen without adjuvant at about days 14, 21 and 63 post primary immunization. At about day three after the final booster immunization individual mice are serologically tested for anti-immunogen antibodies. Spleen cells from antibody producing mice are isolated and fused with murine myeloma cells, such as SP-2/0 or the like, by techniques known in the art (Kohler and Milstein, Nature 256; 495-497, 1975). Hybridoma cells are selected by growth in hypoxanthine, thymidine and aminopterin in an appropriate cell culture medium such as Dulbecco's modified Eagle's medium (DMEM). Antibody producing hybridomas are cloned, preferably using the soft agar technique of MacPherson, (Soft Agar Techniques, Tissue Culture Methods and Applications, Kruse and Paterson, eds., Academic Press, 276, 1973), Discrete colonies are transferred into individual wells of culture plates for cultivation in an appropriate culture medium. Antibody producing cells are identified by screening with the appropriate immunogen. Immunogen positive hybridoma cells are maintained by techniques known in the art. Specific anti-monoclonal antibodies are produced by cultivating the hybridomas in vitro or preparing ascites fluid in mice following hybridoma injection by procedures known in the art.

Detailed Description Paragraph Right (74):

The vaccine according to the invention can be administered in a convential active immunization scheme: single or repeated administration in a manner compatible with the dosage formulation and in such amount as will be prophylactically effective, i.e. the amount of immunizing antigen or recombinant microorganism capable of expressing said antigen that will induce immunity in avians against challenge by virulent Eimeria parasites. Immunity is defined as the induction of a significant level of protection in a population of chickens after vaccination compared to an unvaccinated group.

Detailed Description Paragraph Right (79):
It is clear that a vaccine according to the invention may also contain immunogens related to other pathogens of poultry or may contain nucleic acid sequences encoding these immunogens, like antigens of Marek's Disease virus (MDV), Newcastle Disease virus (NDV), Infectious Bronchitis virus (IBV), Infectious Bursal Disease virus (IBDV), Chicken Anemia Agent (CAA), Reo virus, Avian Retro virus, Fowl Adeno virus, Turkey Rhinotracheitis virus, E. coli or other Eimeria species to produce a multivalent vaccine.

Detailed Description Paragraph Right (84):
A nucleic acid sequence according to the invention can also be used to design specific probes for hybridization experiments for the detection of Eimeria related nucleic acids in any kind of tissue.

Detailed Description Paragraph Right (85):
The present invention also comprises a test kit comprising said nucleic acid sequence useful for the diagnosis of Eimeria infection.

Detailed Description Paragraph Right (101):
20 ml Triton X114 (Serva) was made up to 1 liter with cold TBS pH 7.4 mixed and incubated at 0.degree.-4.degree. C. After complete solubilization the solution was transferred to a 40.degree. C. waterbath. Phase separation was complete after 16 hours. Topphase was removed and replaced by an equal volume of TBS. This procedure was repeated twice. The final bottom phase, called "precondensed TX114", was kept in 100 ml bottle at 4.degree. C. The final TX114 concentration is approximately 20%.

Detailed Description Paragraph Right (111):
Monospecific antibodies were raised in rabbits previously selected for the absence of anti-Eimeria antibodies in the serum.

Detailed Description Paragraph Right (123):
Lane 3 contained the material that did not bind to the 10C-2A column and was thus the starting material for the10E-2 adsorbent. It appeared that this fraction did not contain any Eam45 material. The marked band at 29 kD was artefactual and belonged to the Eam20 protein. The artefact was induced by the presence of Triton X114 in the electrophoresis sample.

Detailed Description Paragraph Right (141):
The lambda gt11 cDNA library was screened with antibodies raised against proteins from Eimeria parasites. Either mouse monoclonal antibodies were used or monospecific rabbit or chicken antisera. Before use the antibodies were diluted in 1.times.Tris-salt (Tris-HCl 10 mM, NaCl 150 mM, pH 8.0)+0.05% Tween 20+10% Foetal Calf Serum (FCS) and incubated for 2 h at room temperature with the filters. The filters were then washed 4 times, for ten minutes each time, with 50 ml 1.times.Tris-salt+0.05% Tween 20 for each filter. For the second antibody incubation a conjugate of goat-anti-mouse or goat-anti-rabbit or rabbit-anti-chicken antibodies and alkaline phosphatase was used (diluted 1:7500 in 1.times.Tris salt+0.05% Tween 20+10% FCS) and incubated for 30 minutes at RT, after which the filters were washed as described above. The colour reaction was carried out in Tris-HCl 100 mM, NaCl 100 mM, MgCl.sub.2 10 mM (pH 9.6) in which 0.33 mg/ml Nitrobluetetrazolium and 0.17 mg/ml 5-Bromo-4-chloro-3-indolyl-phosphate had been dissolved. The reaction was stopped after 30 minutes incubation at room temperature.

Detailed Description Paragraph Right (144):
Antibody select experiments were performed according to Osaki, L. S. et al. (J. Immunological Methods 89, 213-219, 1986) as a final proof for the identity of the proteins the isolated lambda gt11 cDNA clones are coding for. Phagestocks were diluted

to 5.times.10.sup.4 pfu/.mu.l, 1 .mu.l was incubated with 200 .mu.l of cells of E. coli Y1090- and plated. After 2.5 h nitrocellulose filters saturated with IPTG were placed on the plates, after incubation for 5.5 h the filters were turned and the incubation proceeded for another 2 h. The plates with the filters were stored overnight at 4.degree. C., after which the filters were washed with 1.times.Tris-salt for 20 minutes and blocked with 20% FCS in 1.times.Tris-salt for 2 h at room temperature. After a Tris-salt wash for 5 minutes at room temperature the filters were dried at the air. Antibody preparations were purified by caprilic acid precipitation and diluted 1:150 in 1.times.Tris-salt+20% FCS+0.05% NP40. Each filter was incubated with 15 ml of serum for 60 minutes at room temperature. The filters were washed 3.times.for 10 minutes with 1.times.Tris-salt+0.05% NP40. The bound IgG was eluted with 5 ml 0.2M Glycine-HCl (pH 2.8) for 1 minute at room temperature, quickly neutralized with 150 .mu.l 2M Tris, 0.2 ml PBS Tween (25.times.) and 1 ml FCS (all dishes used for the elution steps were first blocked with 1.times.Tris-salt+10% FCS for 1 h at room temperature). The eluates were used on Western blot strips of Eimeria merozoites or sporozoites for identification of the corresponding proteins.

Detailed Description Paragraph Right (147):
Since the initial clones isolated from the lambda gt11library by immunological screening or from the lambda gt10 library by hybridization analysis did not contain the full-length reading frame for the respective proteins additional DNA sequences were generated by the polymerase chain reaction. Towards this end primary cDNA libraries in lambda gt11were amplified: 5*10.sup.4 pfu were incubated with 600 .mu.l E. coli Y1090.sup.- cells and plated. After overnight incubation the top agarose layer was collected in a tube, 5 ml of phage dilution buffer (Tris (pH 7,6) 10 mM,MgCl.sub.2 10 mM,NaCl 100 mM,gelatine 1 mg/ml) were added and incubated for 16 h at 4.degree. C. The suspension was cleared by centrifugation and the supernatant was used directly for PCR reactions. With modifications the method of Blakely and Carman (Bio Techniques 10,53-55 (1991)) was used. To 2.5 .mu.l of the supernatant containing about 10.sup.10 pfu/.mu.l, 1 .mu.l dNTP stock solution (20 mM of each dNTP), 10 .mu.l of buffer (containing Tris 150 mM (pH 7,6), KCl 600 mM, MgCl.sub.2 25 mM), 1 .mu.g of each primer, 3 .mu.l DMSO and 2.5U of Taq Polymerase (Cetus/Perkin-Elmer) was added in a final reaction mixture of 100 .mu.l. One of each primer set is specific for the Eimeria sequence to be extended, i.e. for either Eam20, Eam45 or Eam100; the second primer of each set is a "general" primer, homologous to the 3'-end of the .beta.-galactosidase gene of lambda gt11 (Lambda gt11 Primer (reverse), 24 MER #1222 (New England Biolabs). PCR fragments were purified by gel-electrophoresis and cloned in the vector of the TA-Cloning kit (Invitrogen) exactly according to the instructions of the manufacturer. Resulting clones were sequenced. To correct for PCR-caused errors in the individual DNA clones at least three independent clones for each extended DNA fragment were sequenced.

Detailed Description Paragraph Right (164):
Recognition of Mabs E.ACER 11A-2A (Panel A) and E.ACER 12B-2B (Panel B) on different Eimeria species and stages.

Detailed Description Paragraph Right (165):
Recognition of Mabs E.ACER 10C-2A (Panel A) and E.ACER 10E-2 (Panel B) on different Eimeria species and stages.

Detailed Description Paragraph Left (2):
Triton X114 Extraction

Detailed Description Paragraph Left (3):
Precondensation of Triton X114:

Detailed Description Paragraph Left (22):
Screening of lambda gt11 CDNA library with antisera against Eimeria proteins.

Detailed Description Paragraph Center (5):
Purification of Eimeria antigens

Detailed Description Paragraph Type 1 (1):
1. extracting Eimeria acervulina parasites with a 2% Triton X114 solution,

Detailed Description Paragraph Type 1 (5):
3. 1. eluting the purified 50, 100 or 200 kD Eimeria protein with 0.1M glycine/HCl+0.1% NP40 pH 2.6, or

Detailed Description Paragraph Type 1 (6):
precondensed Triton X114 (TX114) (see below),

Detailed Description Paragraph Type 2 (3):
2. eluting the purified 20 kD Eimeria protein with 3M KSCN in 25 mM Tris/HCl+0.5M NaCl+0.1% NP40 pH 8.0.

Detailed Description Paragraph Table (5):

SEQUENCE LISTING (1) GENERAL INFORMATION: (iii) NUMBER OF SEQUENCES: 10 (2) INFORMATION FOR SEQ ID NO:1: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 1491 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA to mRNA (v) FRAGMENT TYPE: N-terminal (vi) ORIGINAL SOURCE: (A) ORGANISM: Eimeria acervulina (C) INDIVIDUAL ISOLATE: Merozoites (vii) IMMEDIATE SOURCE: (A) LIBRARY: Merozoites cDNA lambda gt11 (B) CLONE: Eam200 (ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 1..1344 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GAATTCGGGGGCACCTCCACTACACACCTGACCCGGGATGATGCAGTG48
GluPheGlyGlyThrSerThrThrHisLeuThrArgAspAspAlaVal 151015
AACACAGCGATTGACTCGAAGCTAGACGAATTCTGCAATCCTACATCA96
AsnThrAlaIleAspSerLysLeuAspGluPheCysAsnProThrSer 202530
GAACCCCCTGAGGCATCGGGAAAGGAGGATTCTGTCGAGGTGGAGGAG144
GluProProGluAlaSerGlyLysGluAspSerValGluValGluGlu 354045
ACAACAACAACCCCACCCAGCCGTCCATTAAGGATGCAACATTTCGTG192
ThrThrThrThrProProSerArgProLeuArgMetGlnHisPheVal 505560
GACGAATTTTGTCTGGAGGAGGCAAAGCGCGCGTGTCAAAATGGGCTG240
AspGluPheCysLeuGluGluAlaLysArgAlaCysGlnAsnGlyLeu 65707580
AGCGCTTACTGCGACGCCAGTGTGAGCGCGCGTCACGACGTGGGAACT288
SerAlaTyrCysAspAlaSerValSerAlaArgHisAspValGlyThr 859095
GAACAGCAGCGGACGAGGGAGTGGCGCTGTTACGTGGATGATTCCCTA336
GluGlnGlnArgThrArgGluTrpArgCysTyrValAspAspSerLeu 100105110
GACTTCGGCCTCTCCGGCGATGGTTGTGTAGACGACTGTGGGAATCTC384
AspPheGlyLeuSerGlyAspGlyCysValAspAspCysGlyAsnLeu 115120125
ATCTCGTGCCCTGGTGCGGTAAACGGCACCTCCACTACACACCTGACC432
IleSerCysProGlyAlaValAsnGlyThrSerThrThrHisLeuThr 130135140
CGGGATGATGCAGTGAACACAGCGATTGACTCGAAGCTAGACGAATTC480
ArgAspAspAlaValAsnThrAlaIleAspSerLysLeuAspGluPhe 145150155160
TGCAATCCTACATCAGAACCCCCTGAGGCATCGGAGAAGAAGGAATCC528
CysAsnProThrSerGluProProGluAlaSerGluLysLysGluSer 165170175
GTCGAGGTGCCAGAGACAACAGCGCTGCCTTCGAACCCCCCATCAAAT576
ValGluValProGluThrThrAlaLeuProSerAsnProProSerAsn 180185190
CTACAAGCTTTGGTGGATGGGCTTTGTGCTGAGGAGGGGAGAAAAGCG624
LeuGlnAlaLeuValAspGlyLeuCysAlaGluGluGlyArgLysAla 195200205
TGCGGACAAGGGCTGCAAGCCTACTGCGACACTGATATGTTCGCACGC672
CysGlyGlnGlyLeuGlnAlaTyrCysAspThrAspMetPheAlaArg 210215220
CACGACGTCGGAACTGGGAGTCAGAGGAACAGGGAGTGGCGCTGCTAT720
HisAspValGlyThrGlySerGlnArgAsnArgGluTrpArgCysTyr 225230235240
GCACGAGTGTCGTTGGACTTCGGCATATCCGGCGATGGTTGTGTAGAC768
AlaArgValSerLeuAspPheGlyIleSerGlyAspGlyCysValAsp 245250255
GACTGTGGGAATCTCACATCTTGCCTTGGTGCGGTAAACGGTTCCTCG816
AspCysGlyAsnLeuThrSerCysLeuGlyAlaValAsnGlySerSer 260265270
ACTACGCATCTCTCACGGGGAGAACGTATTCAAAAACTTATTGACACA864
ThrThrHisLeuSerArgGlyGluArgIleGlnLysLeuIleAspThr 275280285
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AGCCCTGCTCCAGCCCCAGTGCCAGAACTTCCTGCAGGAGTACCGGCG960
SerProAlaProAlaProValProGluLeuProAlaGlyValProAla 305310315320
TCTGAGGTGTCGGACAAGGGCCTGAAGGTTCCTCCAAGGGTCCCAGGT1008
SerGluValSerAspLysGlyLeuLysValProProArgValProGly 325330335
GGTGGAGCTTTACAAGAAATGGCTGACGTCAGGTGCATGGTGTTCTTT1056
GlyGlyAlaLeuGlnGluMetAlaAspValArgCysMetValPhePhe 340345350
GCAAAGCAGTGTGTAACTGACGAAAGCATGTGCCAATACGCCGTGGCC1104
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AlaLysGlnCysValThrAspGluSerMetCysGlnTyrAlaValAla 355360365
CGCAAAATTGACTCCACGTGGAAGTGTTACCCGTATGGTGCAGTTGAT1152
ArgLysIleAspSerThrTrpLysCysTyrProTyrGlyAlaValAsp 370375380
GACTCGCAGTCAGGTGATGCTTGTACAGACGACTGTGGCAATGCAATA1200
AspSerGlnSerGlyAspAlaCysThrAspAspCysGlyAsnAlaIle 385390395400
AACTGTCCGGGTATTCCGAAGAATGGAGATGCCGACGGCATGAGAATT1248
AsnCysProGlyIleProLysAsnGlyAspAlaAspGlyMetArgIle 405410415
CCAGCCCTCGATCACCTGTTCGAAGAGTTGAAGAGCGCCACCTGCAAG1296
ProAlaLeuAspHisLeuPheGluGluLeuLysSerAlaThrCysLys 420425430
ATGAGCAAACAGCAAGAGCTCAAGAAAGTTCACGTGCATCGGCAA1341
MetSerLysGlnGlnGluLeuLysLysValHisValHisArgGln 435440445 TGACGAGAGG1351
GTGTGCTGACTGGACGACGTGGGTTGCGAGGCCAAACTCAATGCTAAGCAAGTGAATGAC1411
AATATAAGTATTCTGCTGCCGGAAGTACTGAAGTCTTCCCTTATCCAATGCAAAGCAAGG1471
CTATCCATGGCCTGGCAGGG1491 (2) INFORMATION FOR SEQ ID NO:2: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 447 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
GluPheGlyGlyThrSerThrThrHisLeuThrArgAspAspAlaVal 151015
AsnThrAlaIleAspSerLysLeuAspGluPheCysAsnProThrSer 202530
GluProProGluAlaSerGlyLysGluAspSerValGluValGluGlu 354045
ThrThrThrThrProProSerArgProLeuArgMetGlnHisPheVal 505560
AspGluPheCysLeuGluGluAlaLysArgAlaCysGlnAsnGlyLeu 65707580
SerAlaTyrCysAspAlaSerValSerAlaArgHisAspValGlyThr 859095
GluGlnGlnArgThrArgGluTrpArgCysTyrValAspAspSerLeu 100105110
AspPheGlyLeuSerGlyAspGlyCysValAspAspCysGlyAsnLeu 115120125
IleSerCysProGlyAlaValAsnGlyThrSerThrThrHisLeuThr 130135140
ArgAspAspAlaValAsnThrAlaIleAspSerLysLeuAspGluPhe 145150155160
CysAsnProThrSerGluProProGluAlaSerGluLysLysGluSer 165170175
ValGluValProGluThrThrAlaLeuProSerAsnProProSerAsn 180185190
LeuGlnAlaLeuValAspGlyLeuCysAlaGluGluGlyArgLysAla 195200205
CysGlyGlnGlyLeuGlnAlaTyrCysAspThrAspMetPheAlaArg 210215220
HisAspValGlyThrGlySerGlnArgAsnArgGluTrpArgCysTyr 225230235240
AlaArgValSerLeuAspPheGlyIleSerGlyAspGlyCysValAsp 245250255
AspCysGlyAsnLeuThrSerCysLeuGlyAlaValAsnGlySerSer 260265270
ThrThrHisLeuSerArgGlyGluArgIleGlnLysLeuIleAspThr 275280285
GluLysAlaGlyArgCysThrProGluGluGlyGluGluAlaGlyGly 290295300
SerProAlaProAlaProValProGluLeuProAlaGlyValProAla 305310315320
SerGluValSerAspLysGlyLeuLysValProProArgValProGly 325330335
GlyGlyAlaLeuGlnGluMetAlaAspValArgCysMetValPhePhe 340345350
AlaLysGlnCysValThrAspGluSerMetCysGlnTyrAlaValAla 355360365
ArgLysIleAspSerThrTrpLysCysTyrProTyrGlyAlaValAsp 370375380
AspSerGlnSerGlyAspAlaCysThrAspAspCysGlyAsnAlaIle 385390395400
AsnCysProGlyIleProLysAsnGlyAspAlaAspGlyMetArgIle 405410415
ProAlaLeuAspHisLeuPheGluGluLeuLysSerAlaThrCysLys 420425430
MetSerLysGlnGlnGluLeuLysLysValHisValHisArgGln 435440445 (2) INFORMATION FOR SEQ ID NO:3: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 944 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA to mRNA (v) FRAGMENT TYPE: N-terminal (vi) ORIGINAL SOURCE: (A) ORGANISM: Eimeria acervulina (vii) IMMEDIATE SOURCE: (B) CLONE: Eam45 M1E (ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 82..489 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
TTTTTTTTTTTTTTGCTCTCCATTTTCCCAACAATATTTCTCTGTTTCTCGTCTTAGGTC60
CGCCTAACCAACATTTAGGAAATGAGTTCGAATCCACGACTCCGGGAAGCC111 MetSerSerAsnProArgLeuArgGluAla
1510 TTTGCCCTTTTCGACAGGGATGGAGACGGAGAGTTGACTGCCAGCGAG159
PheAlaLeuPheAspArgAspGlyAspGlyGluLeuThrAlaSerGlu 152025
GCTCTATTGGCTATCCGTTCTACGGGGGTTATTGTGGCTGCCGAGGAG207
AlaLeuLeuAlaIleArgSerThrGlyValIleValAlaAlaGluGlu 303540
GCAAGCAGCCTGCCGACCACCATGAACTGGGAGCAGTTTGAGAGTTGG255
AlaSerSerLeuProThrThrMetAsnTrpGluGlnPheGluSerTrp 455055
GTCAACAAGAAACTGAGCAGCAGCAACCCGGAGGCGGACTTAATCAAG303
ValAsnLysLysLeuSerSerSerAsnProGluAlaAspLeuIleLys 606570
TCCTTTAAAGTATTTGACACAAAGGGGGACGGCACTCTCTCGACAGAC351
SerPheLysValPheAspThrLysGlyAspGlyThrLeuSerThrAsp 75808590
GAACTTATGCAAGTTATAAAGACCTTAGGAGATCTGCTGACGGACGAA399
GluLeuMetGlnValIleLysThrLeuGlyAspLeuLeuThrAspGlu 95100105
GAGGTTGAGCGTATGGTTAATGACGCAGACCCAAGCAAAACAGGGCGA447
GluValGluArgMetValAsnAspAlaAspProSerLysThrGlyArg 110115120

ATTAAATATGCCGATTTTGTAAAGTACCTCTTGAGCAACTGACTTCATG496
IleLysTyrAlaAspPheValLysTyrLeuLeuSerAsn 125130135
GGTTCATGCAGCACCCCACCACAGCAGTTAAAGCGCTCCTGCTATACTCACGTACATGTT556
GTTCGTGAACGTATGCATGGCTAGGGTTATTTGAACCGCACGGGTTCATTTTGTGCGTTT616
AGTGGAGCCTCTGCCCATCGGGTGCTTCCTCACCTAGCTCTCACAGCAGAGGGCCGAGCG676
CAGGTGTTGCTTTGCCATGGTGCATGTGGGAGTTGCAATCTTTAACCTGCGTGCCGCCTG736
TGTGTTGCTCGCTGCACAGCTGGGGCAGTATTGCATGCACCACATGCATTACGATGGACA796
AAAGACGGGGAGGGGAGCTATGCCTTTCGGTGCTTCTGCCGAGAAAGCGAGCAGCATGCA856
TGCATGTGTGCAACATACATGCGCCAATGTGAGCTATACAACCCCTCCAGGCCTTTTTTA916
TGTGAACGATTTGGAACCGACAAGTCAG944 (2) INFORMATION FOR SEQ ID NO:4: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 135 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
MetSerSerAsnProArgLeuArgGluAlaPheAlaLeuPheAspArg 151015
AspGlyAspGlyGluLeuThrAlaSerGluAlaLeuLeuAlaIleArg 202530
SerThrGlyValIleValAlaAlaGluGluAlaSerSerLeuProThr 354045
ThrMetAsnTrpGluGlnPheGluSerTrpValAsnLysLysLeuSer 505560
SerSerAsnProGluAlaAspLeuIleLysSerPheLysValPheAsp 65707580
ThrLysGlyAspGlyThrLeuSerThrAspGluLeuMetGlnValIle 859095
LysThrLeuGlyAspLeuLeuThrAspGluGluValGluArgMetVal 100105110
AsnAspAlaAspProSerLysThrGlyArgIleLysTyrAlaAspPhe 115120125 ValLysTyrLeuLeuSerAsn 130135 (2) INFORMATION FOR SEQ ID NO:5: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 1631 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear

CLAIMS:

1. A DNA molecule comprising a nucleic acid sequence coding for an Eimeria polypeptide having the amino acid sequence of SEQ ID NO:8, or a fragment of said polypeptide that specifically binds with antibody raised to said polypeptide, wherein the DNA molecule is free from other genetic material of Eimeria.

L3: Entry 3 of 17 File: USPT Mar 20, 2001

DOCUMENT-IDENTIFIER: US 6203801 B1
TITLE: Coccidiosis polypeptide and vaccines

Abstract Paragraph Left (1):
The present invention relates to hydrophilic Eimeria polypeptides, DNA-fragments encoding those peptides, recombinant DNA molecules comprising such DNA-fragments, live recombinant carriers comprising such DNA-fragments or recombinant DNA molecules and host cells comprising such DNA-fragments, recombinant DNA molecules or live recombinant carriers. Furthermore, the invention relates to antibodies against the polypeptides and to coccidiosis vaccines based upon said polypeptides. The invention also relates to methods for the preparation of such antibodies and vaccines, and to methods for the detection of Eimeria parasites and antibodies against Eimeria parasites.

Brief Summary Paragraph Right (1):
The present invention relates to Eimeria polypeptides, DNA-fragments encoding those peptides, recombinant DNA molecules comprising such fragments, live recombinant carriers comprising such fragments or molecules, host cells comprising such fragments, molecules or carriers, antibodies against the polypeptide and coccidiosis vaccines. The invention also relates to methods for the preparation of such antibodies and vaccines, and to methods for the detection of Eimeria parasites and antibodies against Eimeria parasites.

Brief Summary Paragraph Right (2):
Parasitic protozoa belonging to the genus Eimeria are the causative agents of intestinal coccidiosis, an enteritis which affects birds. This causes significant economic loss, especially to the poultry industry. (For the purposes of the present application, the term "poultry" is taken to mean birds that serve as sources of eggs or meat. It includes, inter alia, chickens, turkeys, ducks, geese, guinea fowl, pheasants, pigeons and pea fowl). Nowadays, coccidiosis is mainly controlled by the use of antibiotic drugs in the feed. The rapid emergence of drug resistant strains (Chapman H. D. Parasitology Today 9, 159-162 (1993)) and the prohibitive costs of development and registration of a novel drug have led to increased interest in the development of an alternative method of control. The development of effective vaccines has therefore been desirable for many years. However only partial success has been obtained.

Brief Summary Paragraph Right (3):
Currently available vaccination strategies consist of controlled infections with either virulent or live attenuated parasites (Shirley M. W. In: Proceedings of the Vlth. International Coccidiosis Conference (Eds.: J. R. Barta and M. A. Fernando) Moffitt Print Craft Ltd., Guelph. pp. 61-72 (1993)). For reasons of safety and cost, the most desirable method of immunoprophylaxis against coccidiosis appears to be the use of a subunit vaccine. Although many attempts have been made to immunise chickens against coccidiosis with fractions of parasite material (Murray P. K., Bhogal B. S., Crane M. S. J. & MacDonald T. T. In: Research in Avian Coccidiosis. Proceedings of the Georgia Coccidiosis Conference (Eds.: L. R. McDougald, Joyner L. P. and P. L. Long) Athens, University of Georgia. pp. 564-573 (1986), McKenzie M. E. & Long P. L. Poultry Science 65, 892-897 (1986)) or recombinant Eimeria polypeptides (Danforth H. D., Augustine P. C., Ruff M. D., McCandliss R., Strausberg R. L. & Likel M. Poultry Science 68, 1643-1652 (1989), Jenkins M. C., Augustine P. C., Danforth H. D. & Barta J. R. Infection and Immunity 59, 4042-4048 (1991)) only limited protection against

challenge infection could be achieved. The parasite stages responsible for the induction of protective immunity are generally thought to be early asexual developmental stages (Jenkins et al. 1991). Initially, selection of candidate antigens was performed using antibodies from immune chickens but, in view of the fundamental role of cell mediated responses in protective immunity (reviewed in Lillehoj H. S. & Trout J. M. Avian Pathology 22, 3-31 (1993), Rose M. E. In: Poultry Immunology (Ed.: T. F. Davison, T. R. Morris and L. N. Payne), Carfax Publishing Company, Oxfordshire, U. K. pp. 265-299 (1996), attention has now focused, next to B-cell inducing antigens, on screening antigens for their ability to stimulate specific T-cell responses (Dunn P. P. J., Billington K., Bumstead J. M. & Tomley F. M. Molecular and Biochemical Parasitology 70, 211-215 (1995)).

Brief Summary Paragraph Right (4):
It is an objective of the present invention to provide polypeptides that are capable of inducing protection against the pathogenic effects of Eimeria infection in poultry.

Brief Summary Paragraph Right (5):
It was now surprisingly found that 6 different polypeptides could be specifically identified and isolated, essentially free from other Eimeria polypeptides, from a hydrophilic fraction of Eimeria polypeptides, each of these different polypeptides being capable of inducing an immune response against Eimeria parasites. The inventors have found that these polypeptides can be used, either alone or in combination with each other, to provide a vaccine which gives a significant degree of protection to birds (preferably poultry). For example, protection against the formation of cecal lesions can be achieved in birds immunised with such a vaccine, when subjected to subsequent challenge with Eimeria parasites.

Brief Summary Paragraph Right (6):
A first embodiment of the invention relates to a hydrophilic polypeptide of Eimeria that in its full-length form has a molecular weight of 25 kD and comprises an amino acid sequence that shares at least 70% homology with the amino acid sequence MPFELPPLPYPMDALEPYISKETLEYHYGKHHAAYVNNLNRLVEGKPEASKSLEEIIKTSSGSVLNNAGQAWNH TFYWKSMRPASAGGPPGAPGGGPPGAPGAPLREELESAFGGVEKFREAFAAAAAAHFGSGWAWLCFCKKSRSLFL LQTHDGATPFRDNPNCAPLLTCDLWEHAYYIDRRNDRKSYLDAWWSVVNWDFANENLKKAMQGSD (further referred to as SEQ ID NO: 1:) and immunogenic fragments of that polypeptide capable of inducing an immune response against said polypeptide. The polypeptide is functionally related to a Superoxide Dismutase (SOD) found in non-Eimeria parasites and is therefore characterised as SOD-like.

Brief Summary Paragraph Right (7):
Also, this embodiment relates to a hydrophilic polypeptide of Eimeria that is a peroxidoxin-like polypeptide, in its full-length form has a molecular weight of 22 kD and comprises an amino acid sequence that shares at least 70% homology with the amino acid sequence LGPLALPLLADVR (further referred to as SEQ ID NO: 2:), and immunogenic fragments of the polypeptide capable of inducing an immune response against that polypeptide.

Brief Summary Paragraph Right (8):
A hydrophilic polypeptide of Eimeria that is a peroxidoxin-like polypeptide, in its full-length form has a molecular weight of 25 kD and comprises an amino acid sequence that shares at least 70% homology with the amino acid sequence MPLNLGDSFPDFQAEALGAEHFRLHEYLGDSWGVMFSHPNDFTPVCTTELAEAVKLQDSFTKKNCKLVGFSCND LQSHREWAKDIMAYAGRSGNLPFPLVCDPNRELAASLGIMDPAEKDKKGLPLTCRCVFFISPEKKLAASILYPAT TGRNFAEILRVLDSLQLTAKFPVATPVDWTAGAKCCVVPNLAAEEAQRLLPKGHEALQLPSGKPYLRLTPDPRG (further referred to as SEQ ID NO: 3:), as well as immunogenic fragments of the polypeptide capable of inducing an immune response against that polypeptide are also part of this embodiment.

Brief Summary Paragraph Right (9):
Also part of this embodiment is a hydrophilic polypeptide of Eimeria that in its full-length form has a molecular weight of 22 kD and comprises an amino acid sequence that shares at least 70% homology with the amino acid sequence MSPSPAGVAEYLASL (further referred to as SEQ ID NO: 4:), or an immunogenic fragment of that polypeptide capable of inducing an immune response against said polypeptide.

Brief Summary Paragraph Right (10):
This embodiment also includes a triosephosphate isomerase-like hydrophilic polypeptide of Eimeria that in its full-length form has a molecular weight of 28 kD and comprises an amino acid sequence that shares at least 70% homology with the amino acid sequence NHAEFDPSQTEVVVFP (further referred to as SEQ ID NO: 5:), or an immunogenic fragment of that polypeptide capable of inducing an immune response against said polypeptide.

Brief Summary Paragraph Right (11):
Finally, this embodiment relates to a hydrophilic polypeptide of Eimeria that in its full-length form has a molecular weight of 28 kD and comprises an amino acid sequence that shares at least 70% homology with the amino acid sequence VDSFTPSVGCVFAGMPADFR (further referred to as SEQ ID NO: 6:), or an immunogenic fragment of that polypeptide capable of inducing an immune response against said polypeptide.

Brief Summary Paragraph Right (12):
Although various groups have disclosed Eimeria derived proteins which might, by chance, have molecular masses within the 26-30 kDa.+-.5 kDa range disclosed above, these proteins are quite different from the polypeptides of the present invention.

Brief Summary Paragraph Right (14):
Bouvier et al (J. Biol. Chem. (1985) 260(29); ppl 5504-15509) teach that using Triton X114 extraction amphiphilic proteins (membrane-associated) are only detected in the detergent phase and not in the hydrophilic phase.

Brief Summary Paragraph Right (15):
In U.S. Pat. No. 4710377 (Schenkel et al) antigens are disclosed with molecular masses of about 28 and 26 kDa. However these are amphiphilic outer-membrane components and would not therefore be present in the hydrophilic phase of a Triton X-114 extract which could be used to prepare polypeptides of the present invention.

Brief Summary Paragraph Right (16):
Eimeria proteins that are amphiphilic are also disclosed in W092/04461 (Jacobson et al), EP-A-0324648 (Liberator et al), AU-A-28542/49 (Turner et al), EP-A-0344808 (Alternburger et al) and EP-A-0167443 (Murray et al).

Brief Summary Paragraph Right (17):
It will be understood that, for the particular hydrophilic polypeptides embraced herein, natural variations can exist between individual Eimeria parasites or strains. These variations may exist in (an) amino acid difference(s) in the overall sequence or by deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said sequence. Amino acid substitutions which do not essentially alter biological and immunological activities, have been described, e.g. by Neurath et al in "The Proteins" Academic Press New York (1979). Amino acid replacements between related amino acids or replacements which have occurred frequently in evolution are, inter alia, Ser/Ala, Ser/Gly, Asp/Gly, Asp/Asn, IIe/Val (see Dayhof, M. D., Atlas of protein bsequence and structure, Nat. Biomed. Res. Found., Washington D.C., 1978, vol. 5, suppl. 3). Other amino acid substitutions include Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Thr/Phe, Ala/Pro, Lys/Arg, Leu/lle, Leu/Val and Ala/Glu. Based on this information, Lipman and Pearson developed a method for rapid and sensitive protein comparison (Science,227, 1435-1441, 1985) and determining the functional similarity between homologous proteins. Such amino acid substitutions of the exemplary embodiments of this invention are within the scope of the invention as long as the resulting polypeptides retain their immunoreactivity. Thus, natural variations not essentially influencing the immunogenicity of the polypeptide compared to the wild-type polypeptide, are considered immunologically equivalent variants of the polypeptides according to the invention.

Brief Summary Paragraph Right (27):
Preferably the polypeptide according to the invention is isolated from Eimeria tenella.

Brief Summary Paragraph Right (28):
Another embodiment of the invention relates to DNA fragments encoding a polypeptide of the present invention or immunogenic fragments thereof. Since for the first time the

partial amino acid sequence of the polypeptides according to the invention is now provided, man skilled in the art can (using the genetic code table found in biochemistry textbooks as e.g. in Lubert Stryer's Biochemistry, Ed. Freeman and Company, New York) easily prepare a mixed DNA probe and select the gene encoding the polypeptide according to the invention from Eimeria.

Brief Summary Paragraph Right (29):
There may be minor variations in the overall nucleotide sequence of the DNA encoding the polypeptides according to the invention in the respective Eimeria strains. These variations may have no effect on the amino acid sequence of the polypeptide, in case that the modification is such that the variant triplet codes for the same amino acid. This cause of variation is based upon the phenomenon of degeneracy of the genetic code. It happens e.g. that due to natural mutation the G in the triplet CTG, coding for the amino acid Leucine, is replaced by a C, also coding for Leucine, or that the A in GAA coding for glutamic acid is replaced by a G, which triplet still encodes glutamic acid. Such a mutation is a silent mutation, i.e. it does not show at the amino acid level. Such silent modifications are very frequently found in nature, when comparing e.g. two different field isolates of Eimeria. This phenomenon is found for all amino acids, except Met and Trp. Thus, it is obvious, that the polypeptides of the present invention can be encoded by a very large variety of other sequences, all encoding the identical polypeptide. It therefore goes without saying that any nucleic acid sequence encoding a polypeptide comprising an amino acid sequence that is at least 70% homologous to the amino acid sequence as depicted in SEQ ID NO: 1-6 of the present invention or an immunogenic fragment thereof is also considered to fall within the scope of the invention.

Brief Summary Paragraph Right (30):
Merely for the purpose of giving an example, all possible probes for detecting the gene encoding the 25 kD SOD-like hydrophilic Eimeria polypeptide comprising i.a. the amino acid sequence YLDAWWSVVNWDFANENLK (part of SEQ ID NO: 1:) are given in SEQ ID NO: 7-38. In these SEQ IDs, all possible nucleic acid sequences are listed that code for the amino acid sequence VNWDFA of SEQ ID NO: 1:. Of the 32 probes, one has by definition a perfect fit with each DNA fragment comprising a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence of SEQ ID NO: 1.

Brief Summary Paragraph Right (32):
For the reasons given above, especially a mixed DNA probe encoding the whole amino acid sequence of one of the amino acid sequences given in SEQ ID NO: 1-6 can be used to detect the genes encoding the polypeptides according to the invention in Eimeria.

Brief Summary Paragraph Right (33):
Identification and cloning of the genes encoding the polypeptides according to the invention in Eimeria, not only for tenella but also for the other species, can easily be done as follows: first strand cDNA can be hybridised with both a mixed probe for one of the polypeptides according to the invention and an oligo-dT probe. The DNA fragment between both probes can then be multiplied in a standard PCR reaction. (PCR-techniques are e.g. described in Maniatis/Sambrook (Sambrook, J. Molecular cloning: a laboratory manual. ISBN 0-87969-309-6)). The PCR fragment can then be cloned into a plasmid and e.g. be used for sequencing or for detection of the full length gene in the genome of any Eimeria species.

Brief Summary Paragraph Right (34):
This method allows an easy and straightforward selection and sequencing of the genes encoding the polypeptides according to the invention, not only from Eimeria tenella but also from other Eimeria species such as necatrix, brunette, mitis or acervulina.

Brief Summary Paragraph Right (36):
The mixed probe method described above for the detection of the DNAs encoding the various polypeptides according to the invention has e.g. been used to obtain the DNA encoding the 25 kD SOD-like polypeptide according to the invention in Eimeria tenella. Using the method described in the Examples, a DNA fragment encoding practically the whole 25 kD SOD-like polypeptide of Eimeria tenella could be isolated, cloned and sequenced. The sequence of that DNA-fragment was found to be
ATGCCGTTCGAACTCCCCCCGCTGCCGTACCCCATGGACGCCCTCGAGCCGTACATCAGCAAAGAGACTCTCGA
GTACCACTATGGGAAGCACCACGCGGCTTACGTGAACAACTTGAACAGACTCGTCGAGGGGAAGCCGGAGGCTTC

CAAGAGCCTGGAGGAAATAATAAAGACCTCCTCGGGGTCGGTGCTGAACAACGCGGGCCAGGCGTGGAACCACAC
GTTCTACTGGAAGTCGATGCGGCCGGCCTCGGCGGGGGGCCCCCCGGGGGCCCCCGGCGGGGGCCCCCCGGGGGC
CCCGGGGGCCCCCCTGCGGGAGGAGCTGGAGAGCGCGTTCGGGGGCGTGGAGAAGTTCCGGGAGGCCTTTGCTGC
TGCTGCTGCTGCGCACTTCGGCTCGGGCTGGGCCTGGCTCTGCTTCTGCAAGAAGTCCCGCAGCCTCTTTTTGCT
GCAGACCCACGACGGGGCCACGCCTTTCAGAGACAACCCCAACTGCGCGCCGCTGCTCACCTGCGACCTGTGGGA
GCACGCCTACTACATCGACCGCAGAAACGACCGCAAGAGCTACCTCGACGCGTGGTGGTCTGTGGTGAATTGGGA
CTTCGCGAACGAGAACTTGAAGAAGGCAATGCAGGGAAGCGACTAGGCGCGTGGTGGTCTGTGGTGAATTGGGAC
TTCGCGAACGAGAACTTGAAGAAGGCAATGCAGGGAAGCGACTAG and will be further referred to as SEQ ID NO: 39:

Brief Summary Paragraph Right (38):
The mixed probe method was also used to obtain the DNA encoding the 25 kD peroxidoxin-like polypeptide according to the invention in Eimeria tenella. Using the method described in the Examples, a DNA fragment encoding a large part of the whole 25 kD peroxidoxin-like polypeptide of Eimeria tenella could be isolated, cloned and sequenced. In addition, the genomic sequence, i.e. the sequence of the part of the gene as found in the Eimeria tenella genome was found to be
TTCCCGGATTTTCAGGCGGAGGCGCTGGGCGCCGAGCACTTCCGCTTGCACGAGTACTTGGGGGACAGCTGGGG
AGTGATGTTCAGgtaagattggcgtaaaaaagccccatttaatcgcatttttaattctgtagactctgtgtcgac
tgctgagcacgaggggggggcctgctgcacgggagagccttgtctcgcgctcaactctgggtttctggcgttgct
tgcagCCACCCGAACGACTTCACCCCCGTCTGCACCACCGA. This sequence is further referred to as SEQ ID NO: 40:

Brief Summary Paragraph Right (43):
The polypeptides of the present invention can be isolated from Eimeria parasites using any standard isolation procedure known in the art for isolating Eimeria polypeptides. The polypeptides are e.g. obtainable as described in the Examples. They can be used subsequently for e.g. the preparation of a vaccine or for raising antibodies.

Brief Summary Paragraph Right (48):
When the host cell is yeast, useful expression control sequences include, e.g., .alpha.-mating factor. For insect cells the polyhedrin or p10 promoters of baculoviruses can be used (Smith, G. E. et al., Mol. Cell. Biol. 3, 2156-65, 1983). When the host cell is of mammalian origin illustrative useful expression control sequences include the SV-40 promoter (Berman, P. W. et al., Science, 222, 524-527, 1983) or the metallothionein promoter (Brinster, R. L., Nature, 296, 39-42, 1982) or a heat shock promoter (Voellmy et al., Proc. Natl. Acad. Sci. USA. 82, 4949-53, 1985). Alternatively, expression control sequences present in Eimeria may also be applied. For maximising gene expression, see also Roberts and Lauer (Methods in Enzymology, 68, 473, 1979).

Brief Summary Paragraph Right (59):
Another embodiment of the invention relates to vaccines capable of protecting poultry against the pathogenic effects of Eimeria infection. Vaccines according to the present invention can be made e.g. by merely admixing of a polypeptide according to the invention or an immunogenic fragment thereof and a pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier is understood to be a compound that does not adversely effect the health of the animal to be vaccinated, at least not to the extend that the adverse effect is worse than the effects seen due to illness when the animal is not vaccinated. A pharmaceutically acceptable carrier can be e.g. sterile water or a sterile physiological salt solution. In a more complex form, the carrier can e.g. be a buffer.

Brief Summary Paragraph Right (67):
The vaccine according to the invention can be administered in a conventional active immunisation scheme: single or repeated administration in a manner compatible with the dosage formulation, and in such amount as will be prophylactically effective, i.e. the amount of immunising antigen or recombinant micro-organism capable of expressing said antigen that will induce immunity in birds (especially poultry) against challenge by virulent Eimeria parasites. Immunity is defined as the induction of a significant level of protection in a population of birds after vaccination compared to an unvaccinated group.

Brief Summary Paragraph Right (70):
In any event it is preferred that a vaccine of the present invention is capable of

reducing the number of cecal lesions in a bird when challenged with a subsequent Eimeria infection.

Brief Summary Paragraph Right (72):
The vaccine according to the invention can also be effectively mixed with other antigenic components of the same and/or other Eimeria species, and/or with additional immunogens derived from a poultry pathogenic virus or micro-organism and/or nucleic acid sequences encoding these immunogens.

Brief Summary Paragraph Right (78):
Antibodies or derivatives thereof (e.g. fragments such as Fab, F(ab').sub.2 or Fv fragments), which are directed against a polypeptide according to the invention have potential uses in passive immunotherapy, diagnostic immunoassays and in the generation of anti-idiotypic antibodies. Preferably these are specific for the Eimeria polypeptides of the present invention or variants/fragments thereof. Serum comprising antibodies or derivatives thereof may also be provided.

Brief Summary Paragraph Right (79):
The Eimeria polypeptides (or variants or fragments thereof) as characterised above can be used to produce antibodies, which may be polyclonal, monospecific or monoclonal (or derivatives thereof). If polyclonal antibodies are desired, techniques for producing and processing polyclonal sera are known in the art (e.g. Mayer and Walter, eds. Immunochemical Methods in Cell and Molecular Biology, Academic Press, London, 1987).

Brief Summary Paragraph Right (80):
Monoclonal antibodies, reactive against the Eimeria polypeptides (or variants or fragments thereof) according to the present invention, can be prepared by immunising inbred mice by techniques known in the art (Kohler and Milstein, Nature, 256, 495-497, 1975).

Brief Summary Paragraph Right (85):
It may be desirable to detect Eimeria as the cause of disease in poultry: especially early detection of Eimeria infection in a flock offers the opportunity to take adequate measures for the prevention of spreading of the infection. Detection of Eimeria infection can be done by detecting the Eimeria parasite in the host or by detecting host antibodies against Eimeria.

Brief Summary Paragraph Right (86):
Detection of Eimeria parasites can be done e.g. as follows: DNA prepared from the contents of the digestive tract of a sick animal can be probed with DNA fragments according to the invention and submitted to standard Polymerase Chain Reaction (PCR). If Eimeria DNA is present, even in extremely low amounts, this will result in a PCR-product, visible on standard agarose gels after several rounds of PCR. PCR-techniques are e.g. described in Maniatis/Sambrook (Sambrook, J. Molecular cloning: a laboratory manual. ISBN 0-87969-309-6)

Brief Summary Paragraph Right (87):
Therefore, the invention in still another embodiment relates to methods for the detection of Eimeria, which methods comprise incubating a DNA preparation isolated from poultry with a DNA fragment according to the invention.

Brief Summary Paragraph Right (88):
Alternatively, antibodies against Eimeria can be detected. Detection of antibodies can e.g. be done using an ELISA assay, in which a polypeptide according to the invention is coated to the wall of an ELISA plate. The first step of such an ELISA may e.g. comprise adding serum of the animal to be tested to the ELISA plate. Antibodies against Eimeria, if present at all will bind to the polypeptide coated to the wall. The absence or presence of these antibodies can in a next step i.a. be detected by incubation with a labelled anti-poultry antibody. If antibodies against Eimeria were present in the serum to be tested, the labelled anti-poultry antibody will bind to them and the label will reveal their presence. These standard techniques are extensively described in "Antibodies: a laboratory manual" by Harlow, E. and Lane D. ISBN 0-87969-314-2

Brief Summary Paragraph Right (89):

Therefore, the invention in still another embodiment relates to methods for the detection of Eimeria, which methods comprise the detection of host anti-Eimeria antibodies against any of the polypeptides according to the present invention.

Detailed Description Paragraph Right (3):
Triton-X114 extraction

Detailed Description Paragraph Right (4):
A Triton X-114 extraction was performed to isolate the hydrophilic phase of total sporozoite proteins (HPS) (Bordier C. Journal of Biological Chemistry 256, 1604-1607 (1981)). Hereto, 5.times.10.sup.9 purified E. tenella sporozoites were suspended (2.times.10.sup.8 /ml) in 10 mM Tris-HCl, 150 mM NaCl pH 7.4 (TBS) supplemented with DNAse (20 .mu.g/ml) and protease inhibitors; 1 mM phenylmethyl sulfonyl fluoride (PMSF, Serva, Heidelberg, Germany), 5 .mu.g/ml Aprotinine, 1 .mu.g/ml Leupeptin and 1 .mu.g/ml Pepstatin A and sonified three times 20 seconds at position 7, on ice (using a sonifier from Branson, Soest, The Netherlands). Precondensed Triton X-114 (Serva) in TBS was added to the sporozoite suspension to a final concentration of 10% (v/v) and mixed well to dissolve the proteins. Non-solubilised material was pelleted by centrifugation (20 min 12000 g at 4.degree. C.). The supernatant recovered was layered over a 6% sucrose cushion and incubated 15 min 40.degree. C. (phase separation) and spun 10 min 400 g at room temperature (RT). Extraction of the hydrophilic fraction was repeated once in 10% (v/v) and subsequently in 20% (v/v) precondensed Triton X-114. The total protein concentration was determined using the bichinchonic acid (BCA) assay (Pierce Chemicals, Rockford, Ill., USA). This hydrophilic phase was stored at -70.degree. C. until further use.

Other Reference Publication (3):
Y.D. Karkhanis et al., "Purification and Characterization of a Protective Antigen from Eimeria tenella," Infection and Immunity, vol. 59, No. 3, Mar. 1991, pp. 983-989.

Other Reference Publication (5):
M.C. Jenkins et al., "cDNA Encoding an Immunogenic Region of a 22kilodalton Surface Protein of Eimeria acervulina Sporozoites," Molecular and Biochemical Parasitology, vol. 32, 1989, pp. 153-162.

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☑ 1. Document ID: US 5789233 A Relevance Rank: 99

L3: Entry 12 of 17 File: USPT Aug 4, 1998

US-PAT-NO: 5789233
DOCUMENT-IDENTIFIER: US 5789233 A

TITLE: DNA encoding an Eimekia 50 KD antigen

file 9-21-94

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☑ 2. Document ID: US 5670362 A Relevance Rank: 99

L3: Entry 14 of 17 File: USPT Sep 23, 1997

US-PAT-NO: 5670362
DOCUMENT-IDENTIFIER: US 5670362 A

TITLE: DNA encoding an Eimeria 100kD antigen

file 6-6-95

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☑ 3. Document ID: US 5792644 A Relevance Rank: 99

L3: Entry 11 of 17 File: USPT Aug 11, 1998

US-PAT-NO: 5792644
DOCUMENT-IDENTIFIER: US 5792644 A

TITLE: DNA encoding an Eimeria 200 kd antigen

6-6-95

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☑ 4. Document ID: US 5780289 A Relevance Rank: 99

L3: Entry 13 of 17 File: USPT Jul 14, 1998

US-PAT-NO: 5780289
DOCUMENT-IDENTIFIER: US 5780289 A

TITLE: Coccidiosis poultry vaccine DNA encoding an eimeria 20K antigen

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☑ 5. Document ID: US 5925347 A Relevance Rank: 99

L3: Entry 7 of 17 File: USPT Jul 20, 1999

US-PAT-NO: 5925347
DOCUMENT-IDENTIFIER: US 5925347 A

TITLE: Viral vector vaccines comprising nucleic acids encoding eimeria proteins for poultry vaccination against coccidiosis

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☑ 6. Document ID: US 20020006408 A1 Relevance Rank: 98

L3: Entry 2 of 17 File: PGPB Jan 17, 2002

PGPUB-DOCUMENT-NUMBER: 20020006408
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020006408 A1

TITLE: VACCINES AGAINST EIMERIA MEDIATED DISORDER

PUBLICATION-DATE: January 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
VERMEULEN, ARNO N	CUIJK		NL	
CLERCX-BREED, DOMINIQUE G J	NIJMEGEN		NL	

US-CL-CURRENT: 424/184.1; 424/130.1, 435/320.1, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☑ 7. Document ID: US 6203801 B1 Relevance Rank: 98

L3: Entry 3 of 17 File: USPT Mar 20, 2001

US-PAT-NO: 6203801
DOCUMENT-IDENTIFIER: US 6203801 B1

TITLE: Coccidiosis polypeptide and vaccines

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☑ 8. Document ID: US 20020019358 A1 Relevance Rank: 98

L3: Entry 1 of 17 File: PGPB Feb 14, 2002

PGPUB-DOCUMENT-NUMBER: 20020019358
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020019358 A1

TITLE: Compositions and methods for in vivo delivery of polynucleotide-based therapeutics

PUBLICATION-DATE: February 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Manthorpe, Marston	San Diego	CA	US	
Hartikka, Jukka	San Diego	CA	US	
Sukhu, Loretta	San Diego	CA	US	

US-CL-CURRENT: 514/44

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☑ 9. Document ID: US 6100241 A Relevance Rank: 96

L3: Entry 4 of 17 File: USPT Aug 8, 2000

US-PAT-NO: 6100241
DOCUMENT-IDENTIFIER: US 6100241 A

TITLE: Coccidiosis poultry vaccine

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☑ 10. Document ID: US 5614195 A Relevance Rank: 96

L3: Entry 15 of 17 File: USPT Mar 25, 1997

US-PAT-NO: 5614195
DOCUMENT-IDENTIFIER: US 5614195 A

TITLE: Coccidiosis poultry vaccine

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☑ 11. Document ID: US 5795741 A Relevance Rank: 96

L3: Entry 10 of 17 File: USPT Aug 18, 1998

US-PAT-NO: 5795741
DOCUMENT-IDENTIFIER: US 5795741 A

TITLE: Coccidiosis poultry vaccine

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

☑ 12. Document ID: US 5843722 A Relevance Rank: 96

L3: Entry 9 of 17 File: USPT Dec 1, 1998

US-PAT-NO: 5843722
DOCUMENT-IDENTIFIER: US 5843722 A

TITLE: Coccidiosis poultry vaccine

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

☑ 13. Document ID: US 5885568 A Relevance Rank: 96

L3: Entry 8 of 17 File: USPT Mar 23, 1999

US-PAT-NO: 5885568
DOCUMENT-IDENTIFIER: US 5885568 A

TITLE: Coccidiosis poultry vaccine

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

☑ 14. Document ID: US 6001363 A Relevance Rank: 96

L3: Entry 5 of 17 File: USPT Dec 14, 1999

US-PAT-NO: 6001363
DOCUMENT-IDENTIFIER: US 6001363 A

TITLE: Coccidiosis poultry vaccine

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☑ 15. Document ID: US 2002006408 A1, NZ 330158 A, EP 872486 A1, AU 9860754 A, JP 10298104 A, ZA 9802763 A, CA 2234472 A, BR 9801023 A, MX 9802800 A1 Relevance Rank: 94

L3: Entry 17 of 17 File: DWPI Jan 17, 2002

DERWENT-ACC-NO: 1998-465364
DERWENT-WEEK: 200212
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TITLE: Eimeria sporozoite extract - useful as vaccine against coccidiosis in poultry

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☑ 16. Document ID: US 5997881 A Relevance Rank: 93

L3: Entry 6 of 17 File: USPT Dec 7, 1999

US-PAT-NO: 5997881
DOCUMENT-IDENTIFIER: US 5997881 A

TITLE: Method of making non-pyrogenic lipopolysaccharide or A

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☑ 17. Document ID: JP 10298104 A Relevance Rank: 93

L3: Entry 16 of 17 File: JPAB Nov 10, 1998

PUB-NO: JP410298104A
DOCUMENT-IDENTIFIER: JP 10298104 A
TITLE: VACCINE

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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